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REVIEW ARTICLE

Early life exposure to antibiotics and the risk of childhood allergic diseases: An update from the perspective of the hygiene hypothesis

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The prevalence of allergic diseases has been growing rapidly in industrial countries during recent decades. It is postulated that growing up with less microbial exposure may render the immune system susceptible to a T helper type 2 (Th2)-predominant allergic response—also known as the hygiene hypothesis. This review delineates recent epidemiological and experimental evidence for the hygiene hypothesis, and integrates this hypothesis into the association between early life exposure to antibiotics and the development of allergic diseases and asthma. Several retrospective or prospective epidemiological studies reveal that early exposure to antibiotics may be positively associated with the development of allergic diseases and asthma. However, the conclusion is inconsistent. Experimental studies show that antibiotics may induce the Th2-skewed response by suppressing the T helper type 1 (Th1) response through inhibition of Th1 cytokines and disruption of the natural course of infection, or by

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disturbing the microflora of the gastrointestinal (GI) tract and therefore jeopardizing the establishment of oral tolerance and regulatory T cell immune responses. The hygiene hypothesis may not be the only explanation for the rapid increase in the prevalence of allergic diseases and asthma. Further epidemiological and experimental studies addressing the issue of the impact of environmental factors on the development of allergic diseases and the underlying mechanisms may unveil novel strategies for the prevention and treatment of allergic diseases in the future.

Introduction

During the past two decades, allergic diseases have rapidly grown to afflict more than 20% of the population in industrial countries[1] and are associated with a large percentage of allergy-related comorbidity.[2,3] However, explanations for the rapid and dramatic increase in the prevalence of allergic diseases are still unsatisfying. Hypotheses assume that the phenomenon may result from the emergence of unidentified risk factors or the loss of protective factors in modern lifestyles. Recently, the hygiene hypothesis provides clues to the answers and inspires the research exploring the association between the use of antibiotics and the development of allergic diseases. Numerous studies have reported on the association between early life exposure to antibiotics and subsequent allergic disease.[4–6] However, the results are not completely consistent[7–10] with many debates on the bias of the study design. In this article, we review very recent epidemiological studies and delineate the possible mechanisms from the perspective of the hygiene hypothesis on the basis of current evidence from cellular and animal studies.

Hygiene hypothesis

By means of the successful strategies for controlling infectious diseases in modern society, there has been a great improvement in human health and longevity, and the threats to human health have changed from infectious diseases to chronic diseases. Allergic diseases, such as allergic rhinitis, atopic dermatitis, and allergic asthma, are chronic inflammatory disorders resulting from the complex interaction between the genetic background and the environments. A marked steady increase in the prevalence of allergic diseases has been noticed worldwide during recent decades,[11] causing a great burden on medical resources. It has been observed that the development of allergic diseases is associated with modern lifestyle, and the increase of allergic diseases is paralleled to the decrease of infectious diseases during the same period.[12] The decrease in infectious diseases attributes to multiple factors including the establishment of the public health system, the development of vaccine and vaccination policy, and the use of antibiotics. All of these factors contribute to a much more hygienic living environment. Although the genetic background of humans over past decades is nearly identical, the rapid increase in the prevalence of allergic diseases is most likely associated with changes in environmental factors. The hygiene hypothesis, proposed by Strachan in 1989,[13] provides a possible explanation that the lack of microbial exposure as a result of very hygienic conditions in early life may have an impact on the balance of the immune system, which finally leads to the development of allergic diseases. Although it may not be the sole explanation for the increase in allergic diseases, the hygiene hypothesis has been supported by numerous epidemiological and experimental studies.

Epidemiological evidence for the hygiene hypothesis

The very early epidemiological study by Strachan suggests an association between the prevalence of hay fever/atopic dermatitis and the family size, which means that the higher infection rate of children with more older siblings protects against the development of allergic diseases.[14] In concordance with the family size study, day care attendance in early life reduces the risk for asthma and wheezing in high-risk children[15] and in children without a maternal history of asthma.[16] Epidemiological studies comparing the prevalence in rural and urban areas also provides support for the hygiene hypothesis. Growing up in the farming environment, or even prenatal exposure to such a specific environment, results in protection against the development of atopy, wheezing, and asthma,[17–19] and the conclusion is further supported by identifying the responsible genes with significant interactions with farm exposure for asthma or atopy in the farming environment.[20]

Further evidence for the environmental impact on the development of allergic disorders is from studies comparing the prevalence of allergic diseases in the population with an identical genetic background but with different lifestyles. For example, before the reunification of Germany, the prevalences of asthma, wheezing, and allergic rhinitis were quite low,[21] and a similar condition was also found in other countries of Eastern Europe.[11,22,23] However, the prevalence of allergic diseases rapidly increased after the living condition became westernized.[24] These epidemiological studies have provided evidence for the imprinting effect of infection in early life on the prevention of allergic diseases later in life.

Experimental evidence for the hygiene hypothesis

During pregnancy, the environment of the fetus in the mother is T helper type 2 (Th2)-predominant to protect

the fetus from being rejected. After birth, in very early life, the immune system retains the Th2-predominant response in continuation of the environment of the fetus.[25] In early childhood, the immune system is challenged by infections and gradually deviates to the T helper type 1 (Th1) response, which helps to restore the balance between Th1 and Th2 responses later in life.[26] Because allergy is proposed as a typical Th2-predominant disease,[27] it is postulated that the lack of microorganism exposure in early life prevents the deviation from the Th2 to Th1 response and results in a persistent status of Th2 predominance. In the murine asthma model, early administration of microorganisms, including attenuated live bacteria (Bacillus Calmette Guérin, BCG),[28] killed bacteria,[29] components of bacteria (such as CpG oligodeoxynucleotides),[30] Chlamydia,[31] virus,[32] or even parasite,[33] has preventive or inhibitory effects on the development of allergic diseases and asthma. The cellular and molecular mechanisms accounting for the phenomenon have been postulated from the role of innate and adaptive immunity. In addition to the lack of the Th1 response triggered by infection, the lack of a regulatory immune response that limits the Th2 response in preventing an allergic reaction may also be involved.

The induction of regulatory T cells (Tregs), which produce interleukin (IL)-10 as well as transforming growth factor (TGF)-β and express the costimulatory molecule cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), plays a key role in regulatory immune responses.[34,35] There are two major groups of Tregs, the natural $CD4^+CD25^+$ Tregs (nTregs) and the inducible Tregs (iTregs). Tregs inhibit both Th1 and Th2 responses and are critical for regulating allergic reaction and autoimmunity.[36,37] The inhibitory effect can be antigen-specific or antigen-nonspecific, depending on the types of Tregs.[38] The induction of Tregs is an important one of the mechanisms by which the infections with bacteria[39,40] or helminthes[41] modulate and attenuate the allergic responses and asthma.

In addition to T cells, dendritic cells (DCs) are also important in regulatory immune responses. DCs are professional antigen-presenting cells that induce T cell activation and orchestrate T cell polarization. DCs are heterogenous in phenotype and character, and the function of DCs depends on the production of cytokines, the synthesis of intracellular enzymes, and the expression of costimulatory molecules. DCs have been proven to play an important role in the pathogenesis of allergic diseases.[42] DCs respond to common inhaled aeroallergens[43] and lead to the Th2 response by polarizing the expression of cytokines and costimulatory molecules.[44] We and others have demonstrated that the modulatory effect of some potent anti-asthmatic medications on the function of DCs is an important mechanism to inhibit allergic inflammations.[45,46] The critical role of DCs in the modulatory effects of early life infection on allergic responses has been proposed in recent studies.[47–49] These studies reveal that DCs can be educated by types of microorganisms to become mature and promote the Th1 response that subsequently inhibits the Th2 response of allergy.

DCs can be induced to have a tolerogenic function. The tolerogenic DCs can induce tolerance of T cells by producing IL-10 to induce T cell anergy,[50] by expressing Fas ligand to cause T cell apoptosis,[51] and by inducing differentiation of Tregs. There are several mechanisms by which tolerogenic DCs induce Tregs. Tolerogenic DCs can produce IL-10, TGF-β or interferon (IFN)-α, either alone or in combination, to induce Tregs.[52] The synthesis of intracellular enzyme indoleamine 2,3-dioxypgenase (IDO) in tolerogenic DCs participates in inducing Tregs.[53] The expression of costimulatory molecules, such as OX-2 (CD200) or inducible costimulatory ligand (ICOS-L), in DCs is involved in the development of Tregs.[54]

In contrast to T cells, which use the specific T cell receptors to recognize pathogen, DCs use the pattern recognition receptors, such as Toll-like receptors (TLR), to recognize various types of pathogens. The signaling activated by different types of pattern recognition receptors, mainly TLRs, may influence the expression of cytokines, intracellular enzymes, and costimulatory molecules in DCs. Accordingly, the signaling alters the function and phenotype of DCs and subsequently prevents the development of allergic diseases.[55] The modulatory effects of activated pattern recognition receptors on the function of DCs may be a basis for cellular and molecular mechanisms by which microorganism infection can modulate the allergic response. Studies have reported that DCs from mice infected with bacteria suppress allergic inflammation by increasing IFN-γ and IL-12 (the Th1-related cytokines) and decreasing IL-4, IL-5, IL-9, and IL-13 (the Th2-related cytokines),[48] or by producing IL-10 to induce Tregs.[39] The expression of TLRs is not limited to innate immune cells but can be found in epithelial cells, B cells, or T cells. Interestingly, TLR2 is recently reported to regulate the proliferation of Tregs and affect the suppressive activity of Tregs.[56] These proposed mechanisms by which infection modulate allergic responses via DCs/T cells are summarized in Fig. 1.

Early life exposure to antibiotics and the development of allergic diseases and asthma

Although there were regional variations in prescription rates, the use of antibiotics in early childhood were markedly increased in the late 1980s and early 1990s.[57] This phenomenon coincides with the increase in the prevalence of allergic diseases during the past decades, creating speculation on its causal association. Based on the hygiene hypothesis, it is rational to propose that the increase of early life exposure to antibiotics reduces the exposure of microorganisms and subsequently promotes Th2-predominant allergic immune responses. Therefore, numerous retrospective or prospective epidemiological studies have been performed to evaluate the association between early life exposure to antibiotics and the development of allergic diseases and asthma. We have reviewed and summarized these studies in Table 1, and delineate the mechanisms by which antibiotics modulate allergic responses according to current evidence from *in vitro* and *in vivo* experiments (Fig. 2).

Epidemiologic evidence

Initially, the results from most retrospective studies consistently reveal a positive association between the early

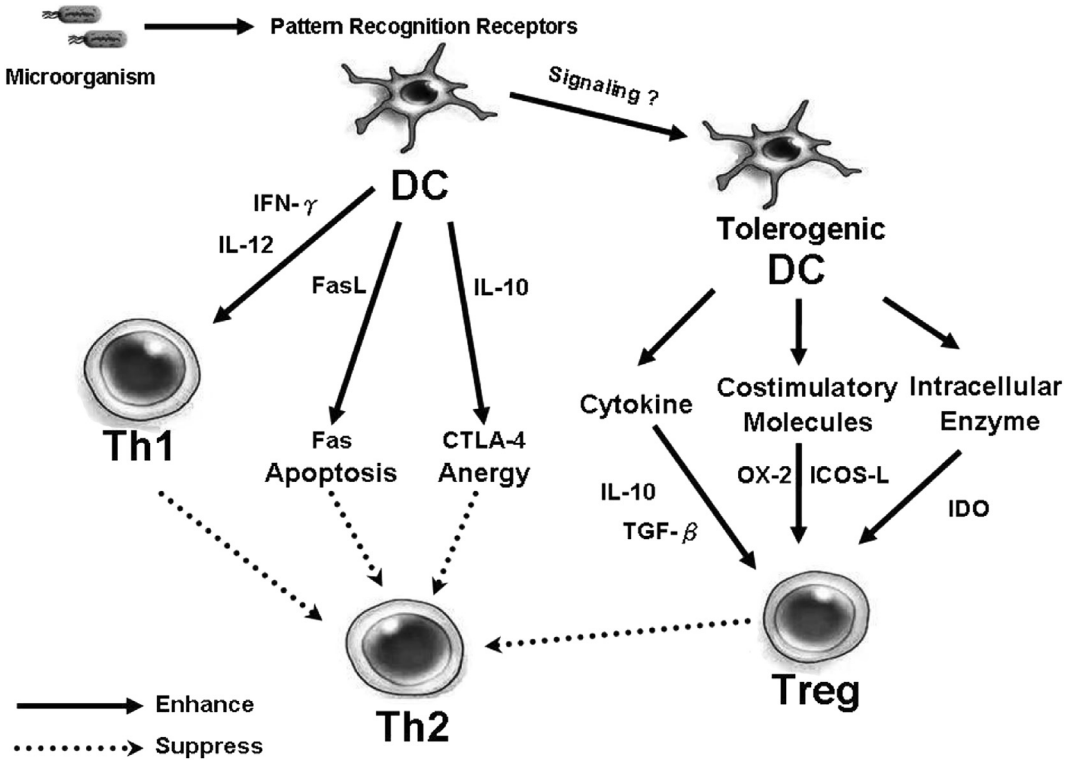

Figure 1. Dendritic cells (DCs) are involved in the modulatory effect of infection on T helper 2 (Th2)-predominant allergic responses. DCs are activated by microorganisms via pathogen recognition receptors and become mature, and promote the T helper 1 (Th1) response by producing interferon (IFN)-γ and interleukin (IL)-12 that subsequently inhibit Th2 responses. DCs also inhibit the Th2 response by producing IL-10 and interacting with the costimulatory molecule cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) of the T cell to induce T cell anergy, or by expressing Fas ligand (FasL) to induce T cell apoptosis. DCs can differentiate into tolerogenic DCs, which can produce IL-10 and transforming growth factor (TGF)-β, synthesize intracellular enzyme indoleamine 2,3-dioxypgenase (IDO), and express specific costimulatory molecules such as OX-2 (CD200) or inducible costimulatory ligand (ICOS-L) to induce regulatory T cells (Tregs), which subsequently inhibit the Th2 response.

life exposure to antibiotics and the development of allergic diseases.[4,5,58] However, the conclusion becomes conflicting in later prospective studies.[7,9] The inconsistence reflects the discrepancy of study design, the methods of analysis, and the adjustment for confounding factors between these investigations.

It is criticized that most retrospective studies require questionnaires that have to be completed by parents, and the results from these studies can be affected by recall bias because parents of asthmatic children may have more medical visits and are more likely to report the early life use of antibiotics. In addition, the diagnosis of allergic diseases and asthma in the retrospective studies may not be ascertained according to just the statement of the parents but rather from objective medical records. In order to verify the hypothesis ideally, prospective birth cohort studies are carried out. In a meta-analysis report by Marra et al,[8] most of the prospective studies cannot yield a positive association,[7,9,59] except for the results from the study by McKeever et al,[10] which enrolled 21,129 subjects and found a positive association between early life exposure to antibiotics and the development of asthma. In case-control studies, the results are also conflicting. Thomas et al[60] report a positive association between antibiotics use within the first years of life and subsequent wheezing as well as atopy, whereas Mullooly et al[61] report a negative association between the early life use of antibiotics and atopy. Even in very recent studies within 5 years, the conclusion is still controversial, regardless of the retrospective or prospective design. The largest retrospective study enrolling 193,412 subjects[62] and the largest prospective study enrolling 251,817 subjects[63] both reveal a positive association. However, others reveal a null association after adjustment for some confounding factors, particular early life respiratory infection[64,65] and medical visits.[66] The most debated issue on the positive association between early life exposure to antibiotics and development of allergic diseases is the protopathic bias, which indicates that the early symptoms of asthma, such as prolonged productive cough, may be the reasons for the use of antibiotics. In a birth cohort study, the association between antibiotic use during the first year of life and development of wheezing and asthma at the age of 4 years is still significant even after adjustment for respiratory infections,[64] but the significant association fades away when analyzing the subgroup of the children without allergic signs in the first year of life. Similarly, in other studies, the association is weaker in children with asthma diagnosed after the age of 3 years than in children with asthma diagnosed earlier.[9] In a large database study from Canada, the age at which

Table 1 Studies investigating the association between early life exposure to antibiotics and the subsequent development of allergic diseases in the pediatric population from 2001 to 2011

Author	Year	Country	Study design	*N*	Age (y)	Conclusion	Ref.
Risnes	2011	US	Prospective	1401	0—6	Antibiotics use within the first 6 months of life is positively associated with asthma and allergy at 6 years	67
Mai	2010	Sweden	Prospective	3306	0—8	Antibiotics within the first year of life is NOT associated with wheezing and eczema after adjustment for respiratory infection	64
Su	2010	US	Prospective	424	0—5	Antibiotics within the first 9 months of life is NOT associated with asthma after adjustment for number of illness visits	66
Garcia-Marcos	2010	Spain	Retrospective	13,908	6—7	Antibiotics use within the first year of life is positively associated with eczema	77
Foliaki	2009	Multiple countries	Retrospective	193,412	6—7	Antibiotics use within the first year of life is positively associated with asthma, rhinoconjuntivitis, and eczema	62
Marra	2009	Canada	Prospective	251817	0—9	Antibiotics use within the first year of life is positively associated with asthma	63
Verhulst	2008	Belgium	Prospective	154	0—1	Antibiotics use within the first year of life is positively associated with wheezing, but the effect is probably due to reverse causation	96
Wickens	2008	New Zealand	Prospective	986	0—4	Antibiotics use within the first 3 and 15 months of life is NOT associated with asthma and atopy after adjustment for respiratory infection	65
Kusel	2008	Australia	Prospective	198	0—5	Antibiotics use within the first year of life is NOT associated with asthma and atopy after propensity score adjustment	69
Alm	2008	Sweden	Prospective	4921	0—1	Antibiotics use in neonatal period is positively associated with wheezing	97
Mullooly	2007	US	Retrospective	1074	6—16	Antibiotics use with the first 2 years of life is NEGATIVELY associated with atopy	61
Kozyrskyj	2007	Canada	Prospective	13,116	0—7	Antibiotics use with the first year of life is positively associated with atopy	68
Thomas	2006	UK	Retrospective	74	3—5	Antibiotics use within the first year of life is positively associated with wheezing and atopy	60
Ahn	2005	Korea	Retrospective	26,400	7—12	Antibiotics use within the first year of life is positively associated with asthma	98
Johnson	2005	US	Prospective	448	0—7	Antibiotics use within the first year of life is positively associated with atopy	99
Celedon	2004	US	Prospective	4408	0—5	Antibiotics use within the first year of life is NOT associated with asthma	9
Cohet	2004	New Zealand	Retrospective	1584	8—9	Antibiotics use within the first year of life is positively associated with asthma	6
Celedon	2002	US	Prospective	448	0—5	Antibiotics use within the first year of life is NOT associated with asthma and atopy	7
McKeever	2002	UK	Prospective	29,238	0—11	Antibiotics use within the first year of life is positively associated with asthma, eczema, and hay fever	10
Wjst	2001	Germany	Retrospective	1149	5—14	Antibiotics use within the first year of life is positively associated with asthma, but the effect is probably due to reverse causation	58

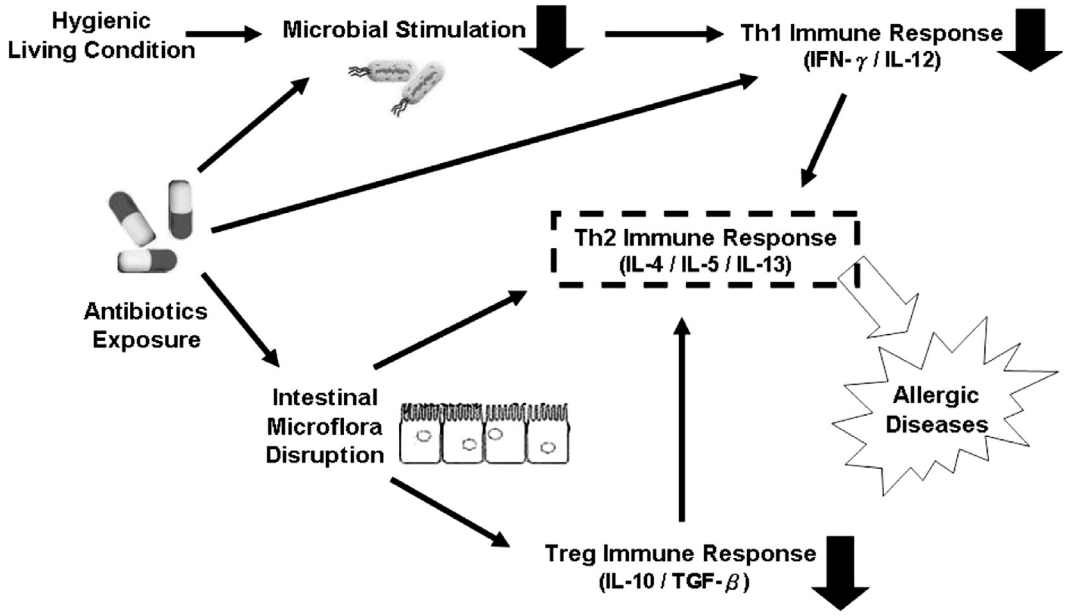

Figure 2. The proposed model for the induction of allergic diseases. Hygienic environments (Westernized lifestyle) and early life exposure to antibiotics result in the decrease of microbial stimulation and the subsequent reduction of the T helper 1 (Th1) response. Meanwhile, the use of antibiotics disrupts intestinal microflora, causing polarization towards the T helper 2 (Th2) response. In addition, the disruption of intestinal microflora by antibiotics suppresses the Treg response, leading to upregulation of the Th2 response. These processes contribute to the deviation towards the Th2 response and the development of allergic diseases.

asthma is diagnosed has no influence on the association.[63] Some studies have several strategies to reduce the bias. For example, in a very recent prospective study,[67] the exposure to antibiotics occurring near the onset of asthma symptoms is excluded, and only the antibiotics used for indications other than asthma-like symptoms is included. The results show a strong association between antibiotics exposure before 6 months of age and asthma diagnosed after the age of 3 years, and this strong association is found particularly in children who did not have lower respiratory tract infection in their first year of life.

The dose—response relationship between early life exposure to antibiotics and the development of asthma supports the conclusion of positive association. Three large prospective studies investigating the connection and the dose—response relationship reveal that the more courses of antibiotics taken during the first year of life, the more likely the risk of developing asthma.[10,63,68] In most studies the information about the types of antibiotics are not well clarified. It is believed that broad-spectrum antibiotics are more potent in reducing microbial exposure and altering microflora in the gut than narrow-spectrum antibiotics, and some studies have verified that the association between early life exposure to broad-spectrum antibiotics and the development of allergic diseases is stronger than that between early life exposure to narrow-spectrum antibiotics and the development of allergic diseases,[10,68] supporting the hypothesis that the effect of antibiotics on the development of allergic diseases is by decreasing the exposure to microorganisms and by changing the microflora of the gut. Among the categories of antibiotics, all kinds of antibiotics are associated with an increased risk of developing asthma but the use of macrolides in particular has the strongest association.[63]

The association of early life exposure to antibiotics and development of allergic diseases and asthma seems more prominent in children without family history of atopy or asthma.[67,68] Many studies have also addressed the relationship between early life exposure to antibiotics and the alternation of immune responses, with methods such as the measurement of immunoglobulin E levels[64,69] and the skin prick test.[4] However, all of these studies do not find a significant association. Because antibiotics do not change the immune response in children with a family history of atopy or asthma, it is reasonable to explain the null association in studies that only include children with a family history of asthma.[7,69]

Mechanism 1—Immunomodulatory effects of antibiotics

Antibiotics with potential anti-inflammatory effects were widely discovered during recent decades. The immune responses induced by infection include the recruitment of inflammatory cells as well as the production of proinflammatory mediators. Some antibiotics, particularly macrolides, are found to exert an anti-inflammatory effect not only by inducing apoptosis of inflammatory cells but also by modulating the production of proinflammatory mediators.[70] Moreover, macrolides inhibit IL-8 expression by human bronchial epithelial cells[71] and prevent neutrophil infiltration into the lung tissue.[72] The key mediators of the Th1 response, such as TNF-α, IL-6, and IL-1β, can be suppressed by macrolides and quinolones.[73] A clinical study reveals that early life exposure to macrolides in particular have the strongest association for the development of allergic diseases.[63] Although there is no direct evidence to prove the

cause-and-effect relationship, it can be speculated that the suppression of Th1 mediators by antibiotics may result in the deviation towards the Th2 response and, accordingly, promote the development of allergic diseases.

Another possible mechanism by which antibiotics suppress the Th1 response is that the use of antibiotics interferes with the natural course of infection. The fever and infection episodes are found to be inversely related to the prevalence of atopy and bronchial hyperresponsiveness.[74] Infections in the first year of life frequently induce fever and the synthesis of IFN-γ.[75] Although IFN-γ is the hallmark of the Th1 response, frequent use of antipyretics and antibiotics may prevent the production of IFN-γ and result in the deviation towards the Th2 response.[76,77] Whether the frequency or the type of the infection can protect the development of asthma is still inconclusive. The proposed mechanism is summarized in Fig. 2.

Mechanism 2—Role of antibiotics in intestinal microbiota and immune tolerance

The gastrointestinal (GI) tract is the largest immune organ and plays a pivotal role in antigen processing and immune regulation. The perturbation of commensal bacteria of the GI tract can be induced by environmental factors[78] and is a common side effect of antibiotics. It is reported that antibiotic use during infancy disturbs the quantity and quality of intestinal microflora and thereby prevents postnatal maturation of the Th1 response, and further results in the deviation towards the Th2 response.[79] Evidence shows that an anthroposophic lifestyle influences the composition of the gut flora, which may contribute to the lower prevalence of atopic disease in children of these families.[80] The normal bacterial microflora in the GI tract generally controls the colonization and growth of fungus, such as *Candida albicans*. Noverr and colleagues found that *C. albicans* and other fungi can secrete prostaglandin (PGD)-like molecules *de novo* or convert exogenous arachidonic acid to PGD.[81,82] Because some PGDs, like PGD_2 or PGI_2, can promote the Th2 response and inhibit the Th1 response,[83,84] the increase of fungi in the gut may upregulate the Th2 response to foreign antigens. Namely, overgrowth of fungi in the microflora of the gut caused by antibiotic therapy may promote Th2 polarization and trigger pulmonary allergic responses.[85]

Although the GI tract serves as a sensor to ingested allergens, the development of oral tolerance has been associated with the modulation of the Th2 response.[86] Oral tolerance helps downregulate the antigen-specific Th2 response in the airway by inducing Tregs.[87,88] It is postulated that a lack of certain bacteria, inappropriate strain colonization, and limited bacterial turnover delay the development of immune tolerance in infancy.[89] The perturbation of the GI microbiota by antibiotics and the delay in the establishment of oral tolerance may cause the defect in the Treg response, resulting in the uncontrollable Th2 response to antigens. The proposed mechanism is summarized in Fig. 2.

The hygiene hypothesis provides a credible explanation for the rapid increase in the prevalence of allergic diseases in industrial countries during recent decades. However, it may not be the sole reason for the phenomenon, and is still facing numerous challenges. For example, some particular inner city populations with poor hygienic living conditions unexpectedly have the highest incidence of asthma.[90] Therefore, studies addressing the issue of the impact of environmental factors, such as early life exposure to antibiotics or the environmental endocrine-disrupting chemicals,[91–93] on the development of allergic diseases and the underlying mechanisms are important. The information may implicate the approaches for treatment or prevention of allergic diseases. A good example is the way to manage allergic diseases by avoiding allergen exposure until the allergen tolerance has been established. Recently, great interest has been focused on the effects of probiotics to treat or prevent allergic diseases.[94] Although antibiotics reduce the exogenous infectious stimuli and disturb the commensal microflora of the intestine, probiotics may offer a safe alternative microbial stimulation. However, current clinical trials do not consistently recommend probiotics as a standard treatment or prevention for the allergic diseases of children.[95] The efforts in exploring the underlying mechanisms may ultimately lead to new strategies for treating and preventing allergic diseases in the future.

Conflicts of interest

All authors declare that they have no conflicts of interest in the manuscript.

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